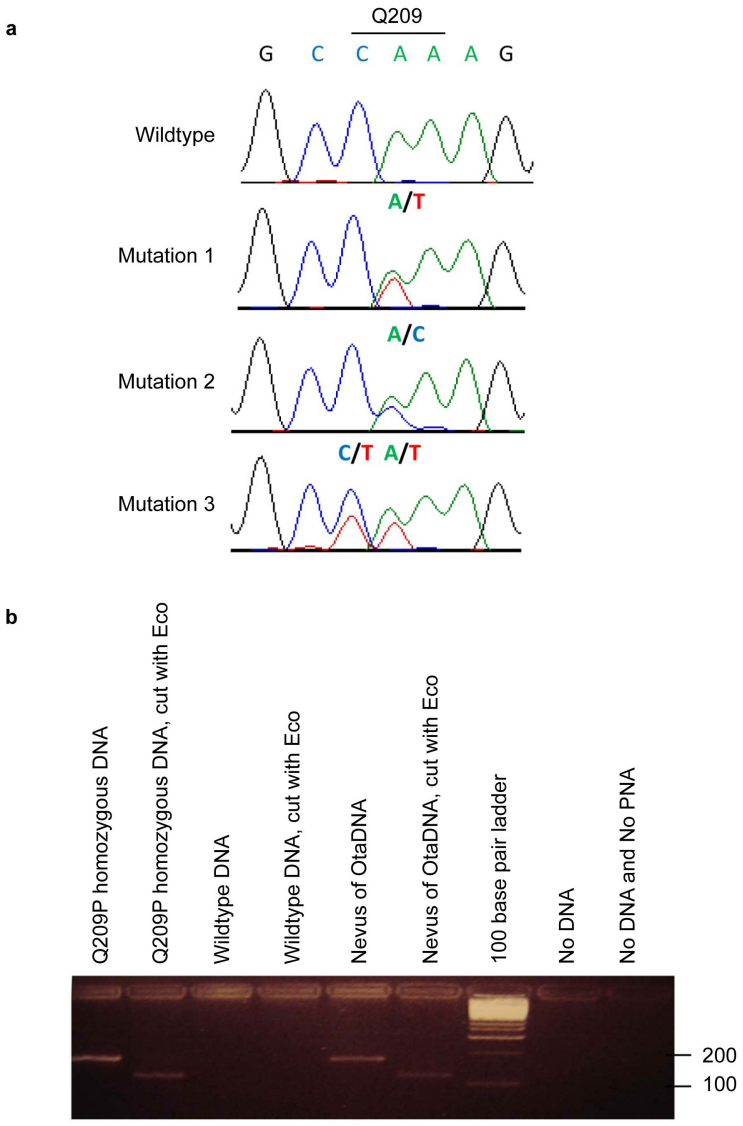
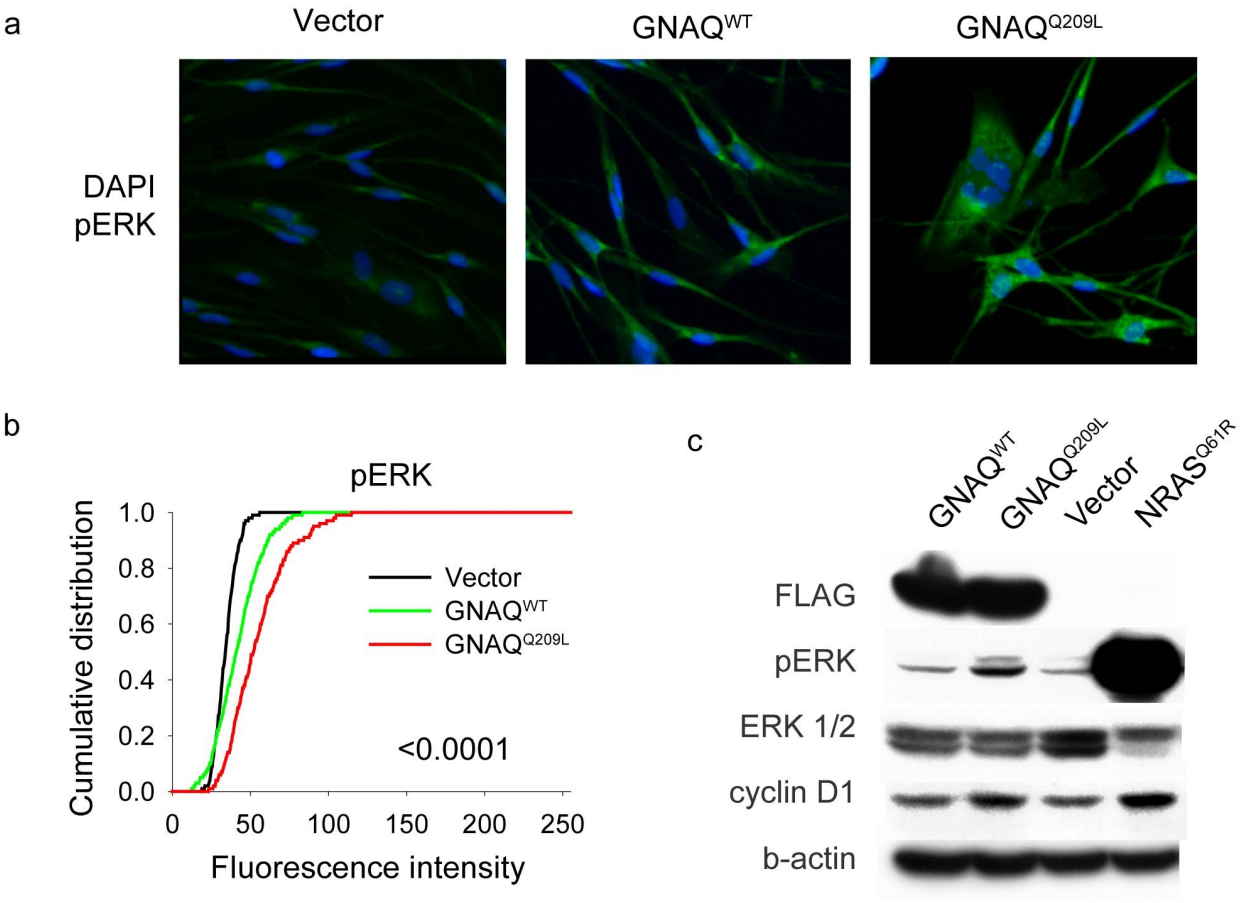
Supplementary Material

1. Supplementary Figures

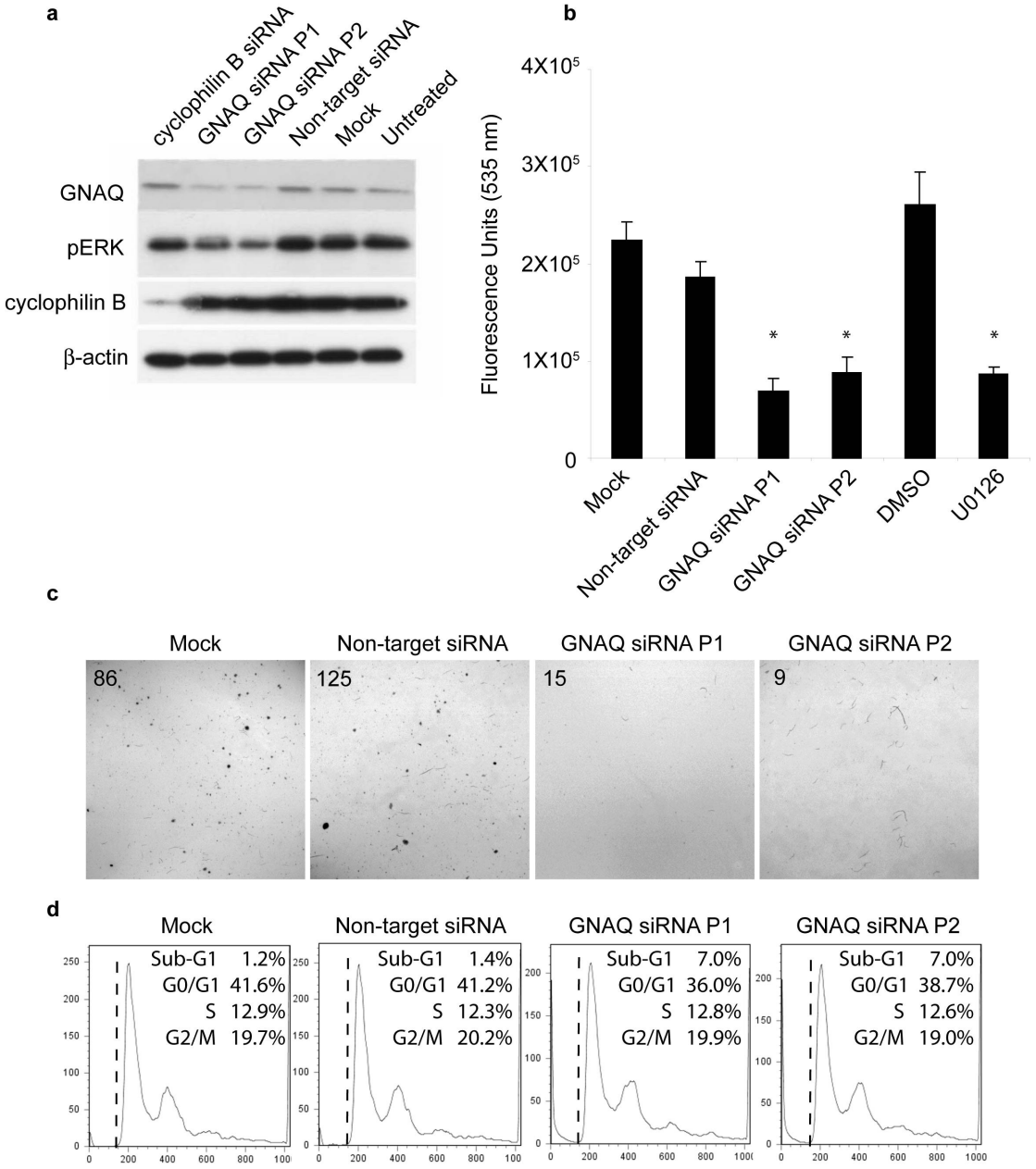


Supplementary Figure 1. Sensitive assay for the detection of GNAQ^{Q209} mutations in Nevus of Ota samples. a. Sequencing traces from representative samples showing the three different mutations found in codon 209 (CAA) in GNAQ. **b.** Nevus of Ota samples are a diffuse mixture of melanocytes and fibroblasts. To detect GNAQ-Q209 mutations in the melanocytes, a 15-mer long PNA (peptide nucleic acid) was designed to span GNAQ codon 209. This PNA hybridizes strongly to the wildtype allele, to which it is complementary, and weakly to mutant alleles with mutations in codon 209. When bound, the PNA prevents the binding of a partially overlapping right PCR primer (which does not include codon 209.) In mutant DNA, a 137 bp product is amplified, which can be digested to 107 and 30 base pairs with one of two restriction enzymes, to confirm that the PCR product did not come from the wildtype allele. Lanes 1 and 2 show successful amplification and cleavage of DNA with a homozygous GNAQ^{Q209P}

mutation, whereas normal genomic DNA is not amplified (lanes 3 and 4). Lanes 5 and 6 show DNA from a nevus of Ota indicating a mutation at codon 209.



Supplementary Figure 2. GNAQ^{Q209L} induces MAP kinase activation in normal human melanocytes and 293T cells. a, Increased expression of pERK in normal melanocytes transduced with GNAQ^{Q209L} and GNAQ^{WT} compared to vector only. **b**, Cumulative distribution of mean pixel fluorescence intensity per cell obtained from immunofluorescent detection of pERK (p-values: GNAQ^{Q209L} vs. vector). **c**, Western blot showing increased pERK and cyclin D1 levels in 293T cells expressing GNAQ^{Q209L} compared to cells transfected with GNAQ^{WT} or vector control. NRAS^{Q61R} transduced 293T cells are shown as a positive control.



Supplementary Figure 3. Knockdown of GNAQ in the GNAQ^{Q209L} mutant uveal melanoma cell line Mel202 results in MAP-kinase inhibition, apoptosis and reduced ability to grow in soft agar. **a**, Western blot analysis of Mel202 cells after treatment with 2 different pools of siRNAs against GNAQ shows decreased pERK levels compared to cells treated with control siRNAs, cyclophilin B and non-target siRNA. **b**, After 72 hours, the siRNAs against GNAQ result in marked reduction of cell numbers, similar to the effect of MEK inhibitor U0126, demonstrated using the CyQUANT cell proliferation assay. (*$p<0.05$, t-test compared to mock or vehicle control, error bars indicate S.E.M.)**c**, siRNA-mediated knowdown of GNAQ reduces the number of colonies (numbers in upper left corner) formed in the soft agar assay.**d**, Cell cycle profiles, including the percentages for each population, showing an increase of the sub-G0/G1 population (dashed line) 72 hr after transfection with GNAQ siRNA compared to cells transfected with non-target siRNA or mock transfection.

2. Supplementary Tables

	Number of samples with mutation				
Diagnosis	**1: CAA(Q) to CTA(L)**	**2: CAA(Q) to CCA(P)**	**3: CAA(Q) to TTA(L)**	**4: CAA(Q) to CGA (R)**	**5. CAA(Q) to TAT(Y)**
CSD Melanoma	1	0	0	0	0
"Malignant blue nevus"	1	0	0	0	0
Blue nevus	17	4	4	0	0
Uveal melanoma	10	10	0	1	1
Uveal melanoma cell lines	2	2	0	0	0
Total	31 (58%)	16 (30%)	4 (8%)	1 (2%)	1 (2%)

Supplementary Table 1. Distribution of the five different mutations found at *GNAQ* codon 209. All mutations result in the substitution of the original glutamine. A RFLP assay was used to confirm that mutation 3 is a tandem base pair mutation of a single allele.

	# of colonies	Average colony size	Std. Dev.	p-value*
Control	334	0.17	0.25	Reference
Vector only	257	0.12	0.10	0.5899
$GNAQ^{WT}$	377	0.16	0.23	0.5134
$GNAQ^{Q209L}$	692	0.38	0.62	<0.0001
$GNAQ^{Q209L}$ w/o TPA	593	0.99	1.35	<0.0001
$NRAS^{Q61R}$	628	0.30	0.35	<0.0001

* Student's t-test for colony size

Supplementary Table 2: Quantitative analysis of colony formation in soft agar.

	N	% abnormal cells	p-value*
Vector	154	7.8	Reference
$GNAQ^{WT}$	172	5.8	0.5140
$GNAQ^{Q209L}$	198	48.9	<0.0001

* Fisher's Exact test.

Supplementary Table 3: Percentage of the cells with enlarged or nuclei with irregular contours.

Type of sample	GNAQ status	Number of samples	% with mutation	
			BRAF	NRAS
Cellular blue nevus	Mutant	11	0	0
	Wild type	2	0	0
Malignant blue nevus	Mutant	1	0	0
	Wild type	1	0	0
Uveal melanoma	Mutant	7	0	0
	Wild type	19	0	0
Conjunctival melanoma	Mutant	0	-	-
	Wild type	10	30%	10%

Supplementary Table 4. Absence of BRAF codon 15 and NRAS mutations in the categories of neoplasms in which GNAQ mutations are frequent.